Pichia pastoris, a methylotrophic yeast is widely used for recombinant protein production. It has a well characterized methanol utilization (MUT) pathway, the enzymes of which are induced when cells are cultured in the presence of methanol. In this study, we have identified an unannotated zinc finger protein, which was subsequently named ROP (repressor of phosphoenolpyruvate carboxykinase, PEPCK) and characterized its function. ROP expression is induced in *P. pastoris* cells cultured in biotin depleted glucose ammonium medium as well as a medium containing methanol as the sole source of carbon. In glucose-abundant, biotin depleted cultures, *ROP* induces the expression of a number of genes including that encoding *PEPCK*. Interestingly, a strain in which the gene encoding ROP is deleted (*ΔROP*) exhibits biotin-independent growth. Based on a number of studies, it was proposed that the ability of *ΔROP* to grow in the absence of biotin is due to the activation of a pyruvate carboxylase-independent pathway of oxaloacetate biosynthesis. It was also proposed that PEPCK, which normally functions as a gluconeogenic enzyme, may act as an anaplerotic enzyme involved in the synthesis of oxaloacetate.

ROP was shown to be a key regulator of methanol metabolism when *P. pastoris* cells are cultured in YPM medium containing yeast extract, peptone and methanol but not YNBM medium containing yeast nitrogen base and methanol. In *P. pastoris* cells cultured in YPM, ROP functions as a transcriptional repressor of genes encoding key enzymes of the methanol metabolism such as the alcohol oxidase I. (AOXI). Deletion of the gene encoding ROP results in enhanced expression of *AOXI* and growth promotion while overexpression of ROP results in repression of *AOXI* and retardation of growth of *P. pastoris* cultured in YPM medium. Subcellular localization studies indicate that ROP translocates from cytosol to nucleus in cells cultured in YPM but not YNBM.

To understand the mechanism of action of ROP, we examined its DNA-binding specificity. The DNA-binding domain of ROP shares 57% amino acid identity with that of Mxr1p, a master regulator of genes of methanol metabolism. We demonstrate that the DNA-binding specificity of ROP is similar to that of Mxr1p and both proteins compete with each other for binding to *AOXI* promoter sequences. Thus, transcriptional interference due to competition between Mxr1p and ROP for binding to the same promoter sequences is likely to be the mechanism by which ROP represses *AOXI* expression *in vivo*. Mxr1p and ROP are examples of transcription factors which exhibit the same DNA-binding specificity but regulate gene expression in an antagonistic fashion.